marker purposes. Together, these results illustrate the development of a new MALDI MSI platform for protein-specific N-glycan analysis from biofluid samples in a clinically-relevant manner requiring minimal sample consumption.

[0217] Described here is a new mass spectrometry imaging (MSI) platform for the multiplexed detection of N-glycans in a protein-specific manner from biological samples. The development of this technique was based on a well-established protocol for enzymatic release of N-glycans from tissue sections for MALDI MSI (Powers T W et al., PloS one, 2014, 9(9), e106255; Powers T W et al., Analytical chemistry, 2013, 85(20), 9799-9806). The two-dimensional analysis with detection by MSI allows for the mapping of N-glycan signals to their carrier proteins along a slide-based antibody array. In this platform, antibodies are essential for the specific capture of glycoprotein targets from a complex biological mixture, similar to an ELISA. Yet unlike an ELISA, no secondary antibody or lectin is needed for this methodology as mass spectrometry provides sensitive and specific detection of distinct N-glycans. Antibody capture also negates the need for sample clean-up prior to MS analysis, which can be extensive (Kailemia M J et al., Analytical and bioanalytical chemistry, 2017, 409(2), 395-410; Kuzmanov U et al., BMC medicine, 2013, 11(1), 31; Song T et al., Analytical chemistry, 2015, 87(15), 7754-7762; Ruhaak L R et al., Analytical chemistry, 2008, 80(15), 6119-6126; Reiding K R et al., Analytical chemistry, 2014, 86(12), 5784-5793). Additionally, typical problems with specificity loss due to heterophilic antibodies present in diseased serum were not observed, which is an important benefit of this technique (Bolstad N et al., Best practice & research Clinical endocrinology & metabolism, 2013, 27(5), 647-661). Antibody capture has been previously used to capture a single target protein for MALDI MS analysis (Darebna P et al., Clinical chemistry, 2018, 64(9), 1319-1326; Pompach P et al., Clinical chemistry, 2016, 62(1), 270-278), however the present novel multiplexed technique can be expanded for the analysis of potentially hundreds or thousands of different N-glycoproteins in one imaging run. Each run generates an immense amount of data, as spectra showing potentially hundreds of N-glycan species are gathered localized to each glycoprotein on the array. Therefore, this method has powerful capabilities for the characterization of N-glycosylation across many target proteins simultaneously.

[0218] This new method extends the capabilities of existing N-glycan biomarker detection technologies. Lectin microarrays have been used for detection of changes in N-glycans in a biomarker setting (Chen S et al., Nature methods, 2007, 4(5), 437; Yue T et al., Molecular & Cellular Proteomics, 2009, 8(7), 1697-1707; Nagaraj V J et al., Biochemical and biophysical research communications, 2008, 375(4), 526-530; Patwa T H et al., Analytical chemistry, 2006, 78(18), 6411-6421), however the present new MSI detection method significantly increases the amount of information that can be obtained from such analyses. While lectins bind to N-glycan structural motifs, MALDI MSI detection provides N-glycans with potential compositional information. The method can be easily adapted to the use of other instrumentation, e.g., ion mobility, which will allow reporting on configuration of N-glycoforms. Additionally, MALDI MSI obtains a complete mass spectrum for each glycoprotein capture spot, allowing hundreds of N-glycan masses to be probed per glycoprotein target as opposed to a select few probed with targeted lectin analysis. Detection of the glycan heterogeneity present on each protein can be used for calculation of glycan ratios, which may represent important alterations in the overall glycosylation of a protein that can be clinically utilized (Callewaert N et al., Nature medicine, 2004, 10(4), 429; Verhelst X et al., Clinical Cancer Research, 2017, 23(11), 2750-2758). As previously mentioned, MSI analyses on tissues have been used for elucidating N-glycan changes in the presence of disease (Powers T et al., Biomolecules, 2015, 5(4), 2554-2572; Kunzke T et al., Oncotarget, 2017, 8(40), 68012; West C A et al., Journal of proteome research, 2018, 17(10), 3454-3462; Scott D A et al., PROTEOMICS—Clinical Applications, 2019, 13(1), 1800014). While tissue-based analysis is often used for prognosis and pathological examination, it is not as an accessible material for early detection of disease, as is serum or other biofluids. The present new biomarker discovery and validation platform is ready for use with readily available patient biofluids such as serum or urine.

[0219] More antibodies can be added so that more glycoproteins can be probed per analysis. This improvement will be limited by the quality of these antibodies—both in binding affinity and specificity. N-glycans present on antibodies can be removed to limit background signal. This technique is applicable to other mass spectrometry platforms for additional structural information of the detected glycans as well as more clinically-accessible MSI instruments. Mass spectrometry imaging of the peptides rather than just N-glycans can be used to confirm glycoprotein binding specificity at each antibody.

[0220] The MSI platform investigated in this study demonstrates its utility as a biomarker discovery tool as well as a new screening platform for a number of diseases in readily available clinical biofluid samples. This platform was able to detect N-glycans on glycoproteins captured from only 1 μL of human serum, illustrating its effectiveness with very minimal patient sample consumption. N-glycans and their role in disease progression are quickly becoming recognized as an important new frontier for biomedical research. However, the applications of this new technique extend beyond just N-glycans and biofluid samples: this platform could be used with liquids such as cell supernatants or probe other classes of glycans or post-translational modifications.

[0221] The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

What is claimed is:

1. A method for glycan analysis of at least one sample, the method comprising the steps of:

providing a substrate having a surface spotted with a plurality of antibodies;

incubating the substrate in a blocking solution;

incubating the substrate in at least one sample;

spraying the substrate with an enzymatic releasing solution; and

scanning the substrate by mass spectrometry to detect and identify the presence of glycans.